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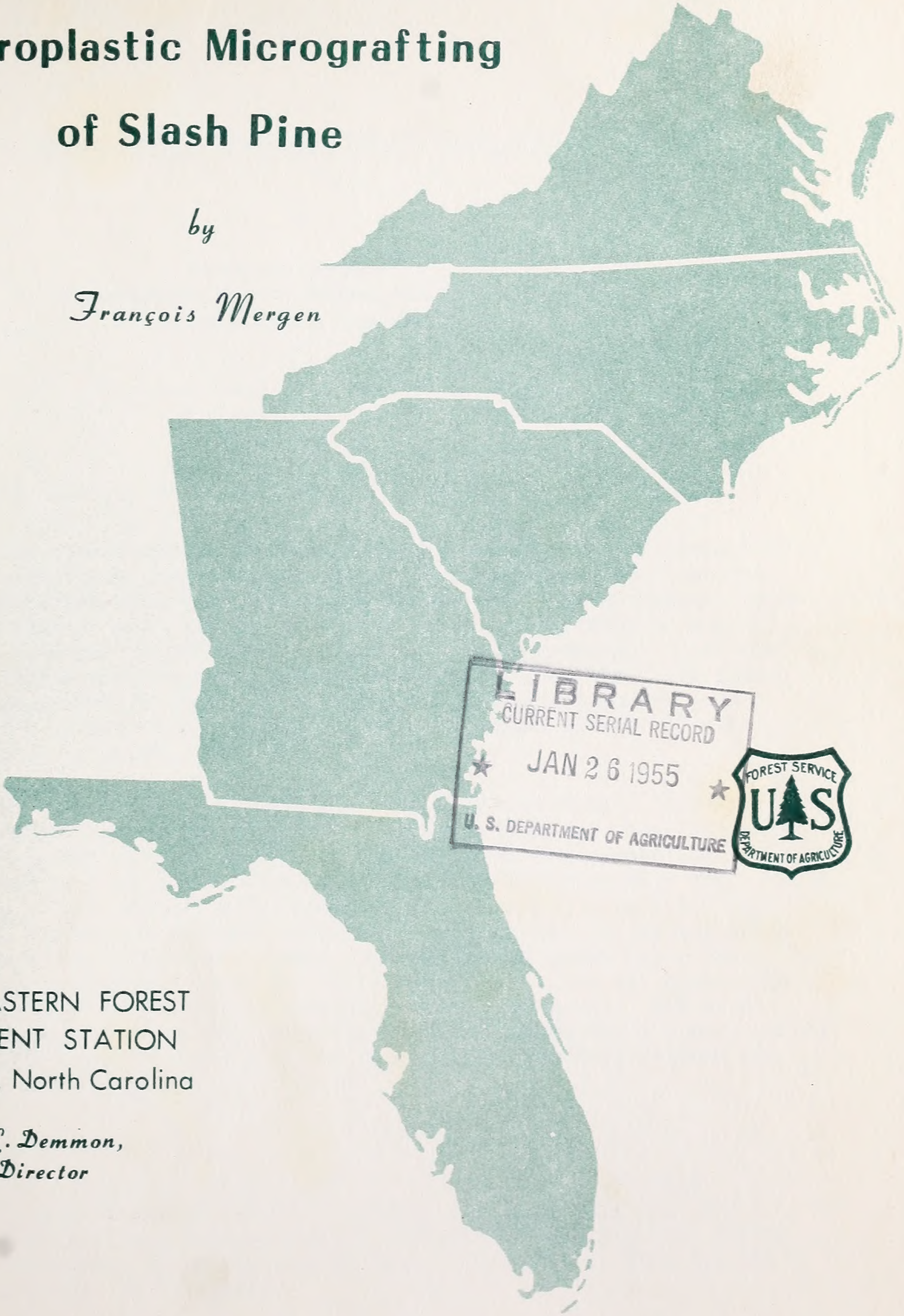

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HETEROPLASTIC MICROGRAFTING OF SLASH PINE

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INTRODUCTION

The time interval between successive generations of slash pine hybrids presents a great problem to the tree breeder. Under natural conditions slash pine trees flower at the age of 10 to 15 years. Occasionally flowers are produced at an earlier age. In order to shorten the time required to produce a new progeny, it is necessary to obtain the pistillate and staminate flowers as early as possible. The iniation of reproductive organs is stimulated by certain hormones, and their further development is controlled by a proper balance of the assimilation products.

In this study an attempt was made to induce flower formation in young seedlings by grafting succulent slash pine seedlings on seedlings of another species, genus, or family. Grafting upsets the harmonious transfer of plant solutes, and this interference with the normal metabolic process might induce flower production in slash pine seedlings during the succulent stage. As no information was available on the possible intergeneric graft combinations of slash pine, the first objective of the study was to find out whether or not slash pine seedlings could be grafted on seedlings of another genus. The main objective, however, was the early formation of reproductive organs during the primary stage of slash pines to shorten the time span between two consecutive generations.

1/ This article is based on part of a dissertation entitled "Rooting and Grafting of Slash Pine (*Pinus elliottii* Engelm.) for Application in Forest Tree Genetics," submitted to the Graduate School of Yale University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

REVIEW OF LITERATURE

In grafting, a scion is transplanted on a new plant and it eventually replaces the "crown" of the stock. Its needles carry on photosynthesis and the stock plant is supplied with these carbohydrates. The stock supplies the necessary water and mineral elements, but it is entirely dependent upon the scion for its organic materials. This form of plant development presents a typical case of artificial symbiosis (11).

The method of grafting is called micrografting when the stock and scion are young succulent seedlings in or recently past the cotyledon stage (18).

Natural grafts between individuals of the same species are occasionally observed and some heteroplastic natural grafts have been reported. Derickson (2) described a union between a member of the rose family and a member of the beech family. A case of a graft between the stem of a dicotyledonous vine and that of a monocotyledon was also reported by Herbert (3), but there were no vascular connections.

The rootstock on which a scion is grafted influences the scion in many ways. It exerts a marked influence on its growth rate (19), affects the age at which the first flowers are produced (10), determines susceptibility to certain tree diseases (14) and controls the concentration of chemical constituents in the plant solutes (15). Roach and Bolas (16) presented results of the effect of rootstock on the mineral composition of the leaves of an apple tree. A large number of minerals, such as calcium, magnesium, potassium, iron, and strontium, varied in concentration with the various rootstocks.

The "ripeness-to-flower" was described by Klebs (7) as a distinct physiological stage in the flowering of higher plants. The shortening of this stage is of great importance to plant breeders. A certain variety of sweet potato does not flower normally in the United States, but recently some horticulturists have been able to induce flowering by heteroplastic grafting (6). Flowering occurred only when the stock plant was a species with non-storage-type roots. The authors concluded that the flower development was associated with an accumulation of reserve materials in the parts above the ground. Herrmann (4, 5) reported a sole instance of male flower production on a 15-month-old *Pinus montana*. This premature catkin was formed by grafting the *P. montana* seedling on a *Picea excelsa* seedling. He postulated that the reason for this phenomenon might be the result of an accumulation of assimilation products above the union as a result of disharmony. He did not exclude possible effects of other factors.

EXPERIMENTAL METHODS

Young slash pines ranging between 1.5 and 6 months in age were grafted on 11 different rootstocks. The attempted graft combinations are summarized in table 1. The original plan called for 10 grafts of each combination. The supply of rootstock was insufficient for some combinations, while a surplus of *Chamaecyparis obtusa* (Sieb. and Zucc.) Endl., and ponderosa pine was on hand.

Most of the seedlings used for grafting stock were raised in the Olustee Experimental nursery and at the time of grafting were 4 months old. The Norway spruce, white spruce, European larch, *C. obtusa*, and white pine seedlings were obtained from commercial nurseries, and were at least 2 years old at the time of grafting. Attempts to grow the northern species from seed in the nursery failed, possibly as a result of the high summer temperatures. The potted seedlings were moved into the greenhouse during the middle of December to stimulate active root growth. The seedlings for scion material were started at periodic intervals in veneer plantbands, so that they could attain suitable sizes for the various graft combinations. The first grafts were attempted during the middle of January.

Grafting on 6-month-old understock was a very precarious operation, as in many instances the stock plant was less than 1 mm. in diameter at the ground line. Some of the seedlings were still in or just past the cotyledon stage (fig. 1). The procedure used in the micro-grafting of the young slash pines of the 6-month-old conifer seedlings was as follows: The slash pine seedling used as the scion was cut off about one inch above the ground line and dipped immediately into a beaker of tap water. With several drops of water adhering to the hypocotyl, the lower one-third of the stem was cut into a wedge shape with a razor blade. An incision was made in the hypocotyl of the stock. The cut was located just below the cotyledons and was slanted inward until the center of the hypocotyl was reached, thence parallel to the axis of the stem and just long enough to permit the complete embedding of the wedge-shaped part of the scion. Holding the cut open with a fine glass needle, the scion was pushed into the side slit and was tied in place with waxed nylon thread. With a camel hair brush, grafting wax was spread over most of the graft. A small strip along the back of the union was left uncovered, leaving the binding exposed along the opposite side of the union to permit observation of growth of the stem.

A layer of moist peat moss was placed around the base of the stock to provide a good seal for the glass jars which were placed over the finished grafts. They were kept for 3 weeks in a cool shaded place in an unheated building before they were moved into the greenhouse. The glass of the greenhouse had been whitewashed to exclude direct

Table 1.--Summary of graft combinations attempted

Stock	Age of stock	Age of scion	Type of graft	Grafts attempted
	Months	Months		Number
Slash pine, Pinus elliottii Engelm.	4	2	Side slit	2
" "	4	1	Saddle	1
" "	4	4	Veneer 1/	7
Loblolly pine, Pinus taeda L.	4	2	Side slit	2
" "	4	2	Saddle 2/	1
Pitch pine, Pinus rigida Mill.	4	1.5	Saddle 3/	1
Ponderosa pine, Pinus ponderosa Laws	4	2	Side slit	12
" "	4	1.5	Cleft	1
" "	4	1.5	Side slit	1
" "	4	1.5	Saddle	1
" "	4	2	Saddle	3
Sand pine, Pinus clausa (Chapm.) Vasey	4	2	Whip	1
White pine, Pinus strobus L.	2	2	Cleft	2
White spruce, Picea glauca (Moench) Voss	2	2	Cleft	5
Norway spruce, Picea abies (L.) Karst	2	2	Cleft	4
European larch, Larix decidua Mill.	2	2	Cleft 4/	3
Douglas-fir, Pseudotsuga menziesii (Mirb.) Franco	4	1	Side slit	2
" "	4	1.5	Saddle 5/	2
" "	4	2	Saddle	2
" "	4	2	Side slit 6/	2
Chamaecyparis obtusa, (Sieb. and Zucc.) Endl.	24	4	Cleft 7/	3
" "	24	1.5	Cleft	1
" "	24	1.5	Saddle	1
" "	24	2	Whip	1
" "	24	4	Side slit	6
" "	24	6	Veneer	1
Total				68

1/ Some of the grafts were covered with plastic sausage casings.
2/ Stock 1 mm. in diameter.
3/ Stock plant less than 1 mm. in diameter.
4/ Stock died after 3 months.
5/ Stock 1 mm. in diameter.
6/ Stock plants were less than 1 mm. in diameter.
7/ Plants covered with plastic sausage casing.

Figure 1.--Completed micrograft of 1-month-old slash pine on Douglas-fir seedling (photographed 3 weeks after grafting).

insolation. The glass jars were kept in place for 3 weeks, at which time they were removed for short periods each day. They were completely removed after 8 weeks. The speed of removal depended somewhat on the type of graft combination and growth rate of the scion. As soon as the growth of the stem started to embed the ties, they were severed with great care.

Whenever the initial cut into the stock plant went too deep, the decapitated stock was prepared for either a saddle, cleft, or whip graft. The typing and waxing were the same for these grafts as for those described above. A magnifying glass mounted on a stand was helpful to gauge the cuts in micrografting.

When slash pine scions were grafted on 2-year-old stock, a cleft or a slit method was used, depending on the location of the union. These grafts were also covered with waterproof transparent containers to reduce transpiration. In some instances where the grafts were too large to be covered by available jars, a plastic sausage casing, closed at one end, was placed over the plant.

The gradual removal of the foliage from the stock plant varied with the various graft combinations. Loblolly pine stock showed the fastest recovery from the grafting shock, and after 5 days the tropistic movement of the top started to push the scion aside. In most cases the foliage of the stock above the union had been removed 2 months after grafting.

During the middle of May the successful grafts were moved from the greenhouse into a shade house and kept under partial shade during the hot part of the early summer. A wire support was placed next to each graft for protection against wind-breakage.

RESULTS

The number of successful unions alive 1 year after grafting varied widely with the different graft combinations. In some instances excellent results were obtained. Table 2 summarizes the established grafts of slash pine with other species. A large number of graft combinations became established but died at various intervals. In many instances death of the stock caused failure. For instance, the needle buds of European larch were fully open during the middle of February, but all plants died during the warm summer months. Even ungrafted plants of larch, which were kept in a shaded place, were unable to live through the hot part of the summer.

Most of the scions grafted on Chamaecyparis obtusa survived for several months and added some growth, but were not able to survive after the jars were removed. One scion survived for a period of 8 months. The growth of several graft combinations is discussed in the photo captions on the following pages.

Table 2.--Summary of successful graft combinations with slash pine scions, 1 year after grafting

Stock	Attempted grafts	Successful grafts	Average height of scions
	Number	Number	Inches
Slash pine	10	9	8.3
Loblolly pine	3	3	6.5
Pitch pine	1	1	5.6
Ponderosa pine	18	8	5.0
White pine	2	1	4.0
Douglas-fir	8	2	4.2
White spruce	5	4	3.5
Norway spruce	4	3	2.2

Figure 2.--Slash pine on slash pine, photographed 4 months after grafting. The seedlings grafted on slash pine ranged from 1 to 4 months in age. Growth of the scion started right after grafting, and normal development continued throughout the growing season. The height of the scions ranged from 4 to 10.3 inches within a year after grafting. The wound healing was very rapid and in several instances the place of union was almost indiscernible. In one case, however, where the scion was only 4 inches tall after 1 year, a faulty union was present. From this faulty union two root primordia were growing from the base of the scion. The color change of the foliage during the winter months proceeded at the same rate as that of the seedlings in the nursery.

Figure 3.--Slash pine on ponderosa pine, photographed 4 months after grafting. Grafting of 1.5- to 2-month-old seedlings on very small ponderosa stock resulted in 80-percent success. After a period of 12 months, only 50 percent were alive. Despite the large number of successful unions, the subsequent growth of the scion was slow, ranging from 3.5 to 6.5 inches within a year. The tissues were very tender and were severely attacked by insects. In many cases the foliage dwindled during the fall and only a small tuft of needles remained by December. The foliage turned rusty in the early part of October, and in many cases no green foliage was left on the scion. Under north Florida conditions slash pine seedlings turn rusty during the winter months, but the grafts changed color at least 8 weeks before ungrafted seedlings or seedlings grafted on slash pine stock.

Figure 4.--Slash pine grafted on eastern white pine, photographed 2 months after grafting. Of the two grafts attempted, one graft developed into a successful combination. The union between the two symbionts initially healed over slowly but after 10 months callus growth had completely covered the wound. The scion was 4 inches high at the end of the first growing season, had a vigorous appearance, and the foliage turned color several weeks before natural slash pines did.

Figure 5.--Slash pine grafted on white spruce, photographed 2 months after grafting. Four out of five attempts to graft 2-month-old seedlings on white spruce stock survived. The unions were successful but the growth of the scion was very slow. The height of the scions after the first growing season ranged from 2.5 to 5.5 inches. The development of secondary needles was retarded so that two of the seedlings still had their primary needles after the first growing season. The foliage changed color very early during the autumn. In one graft combination the knitting of the union was poor and bridging of the layers was apparent only on one side. The other side had died. A rootlet 5 mm. long had developed at the place of union and had started to grow downward.

Figure 6.--Slash pine grafted on Douglas-fir, photographed 4 months after grafting. Eight slash pine scions, ranging in age from 1 month to 2 months, were grafted on Douglas-fir stock 1 mm. thick. Six of these combinations survived for 2 months, at which time four of the stock plants died. At the end of the growing season there were two grafts still alive. Those alive were grafted when the seedlings were 1 month old. The union between the symbionts was strong and well callused over. One of the scions had both primary and secondary needles, while only primary needles had developed on the other graft. Heights of the scions were 3.0 and 5.4 inches. Color change of the foliage took place early in October.

Figure 7.--Slash pine grafted on Chamaecyparis obtusa, photographed 4 months after grafting. Out of a total of 12 attempts, 3 grafts lived for 6 weeks and 1 graft survived for a period of 8 months. The foliage of the scion dried out and withered away. The graft unions were very poor and possibly did not supply enough water to the scion.

The results clearly indicate that heteroplastic grafting of succulent slash pines is physiologically possible. In many cases the failures were the result of mechanical difficulties or inability of the stock plant to live under the subtropical climate of Olustee, Florida. Flower formation in the primary stage was not observed. None of the grafts flowered within a year's time after grafting.

DISCUSSION

Intergeneric and intrageneric grafting of young slash pine seedlings is physiologically possible. The large number of successful graft combinations was probably the result of grafting at an early developmental stage. The young scion material was succulent, no lignification of the tissues had taken place, and most cells were still in an active meristematic stage.

None of the grafts flowered during the first year after grafting. If flower formation can be brought about by heteroplastic grafting, one of the many combinations attempted should have succeeded. The early flowering, however, is not always dependent upon the type of rootstock. Theoretically, any union which restricts the normal translocation of plant solutes can stimulate flowering. Krenke (9) illustrated this point by giving an example of an experiment with *Diospyros kaki* L., in which grafting *per se* influenced flowering. This species produces flowers at the age of 8 to 10 years. When grafted on *Diospyros lotus* L. stock, it flowered in the second or third year. However, a similar reduction of time necessary to attain the "ripeness-to-flower" stage was obtained by an autoplastic graft of *Diospyros kaki* L. In this case, the graft had an effect on metabolism similar to that obtained by partially girdling the stem. Partial girdling or ringing has been used successfully in orchard management to induce the formation of fruits at points above the girdle (17). The single observation of a staminate flower on the 15-month-old *Pinus montana* graft which was reported by Herrmann (4, 5) might not have been the result of the heteroplastic nature of the graft, but could be due to the effect of the graft union itself. Of a large number of grafts and graft combinations which Herrmann tested, early flower production was obtained in one instance only.[2/] This may have been a botanical curiosity.

2/ Personal correspondence with Herrmann, 1953.

The foliage of the scion partners, grafted on the rootstock of another species or genus, turned rusty early during the winter season. This color change is due to anthocyanin and related substances. The production of these pigments is influenced by internal as well as external characteristics. In plant nutrition, the reddish pigmentation of the foliage can be an indication of a nitrogen deficiency (1). Another important factor regulating anthocyanin production is the carbohydrate content of the foliage. Molisch (11) concluded from observations on mechanically injured grapevines that the large amount of carbohydrates which had accumulated above the girdle caused an early and strong anthocyanin formation. A high starch concentration is frequently present above a graft union (12). The rusty color of some of the scions probably indicated a high C/N ratio in the foliage. If this was the case, the nutritional factors for abundant flowering were favorable according to Kraus and Kraybill (8), but the factor or factors which tip the balance to transform vegetative growth to flower formation (13) were not present.

The grafts will be kept under observation to see when the first flowers are formed. It is possible that heteroplastic grafting--although not suitable to induce flowering in 1- to 2-year-old seedlings--can induce premature flowering.

CONCLUSION

The premise that early flower formation can be brought about in young slash pine by heteroplastic grafting was neither proved nor disproved by this study. No signs of flowers had developed after 1 year, but it should be most interesting to follow the growth of these grafts in the next few years. The fact that a large number of scion-stock combinations was successful presents a good basis for future research along this general line. The micrografting technique used in this experiment should also be of interest to students of plant nutrition and plant metabolism.

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